Gap junctions and motor behavior

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The production of any motor behavior requires coordinated activity in motor neurons and premotor networks. In vertebrates, this coordination is often assumed to take place through chemical synapses. Here we review recent data suggesting that electrical gap-junction coupling plays an important role in coordinating and generating motor outputs in embryonic and early postnatal life. Considering the recent demonstration of a prevalent expression of gap-junction proteins and gap-junction structures in the adult mammalian spinal cord, we suggest that neuronal gap-junction coupling might also contribute to the production of motor behavior in adult mammals.

Gap-junction channels connect the interior of two neighboring cells directly. The channels allow ions and small molecules to be exchanged between cells, providing a low resistance pathway for electrical and chemical intercellular coupling. It is generally accepted that this intercellular coupling plays a key role for normal synapse and circuitry formation during early development of the vertebrate nervous system [1,2]. The role of gap-junction coupling (GJC) for the production of brain functions, particularly in postnatal and mature vertebrates, including mammals, is, however, not well understood. One place where this issue might most clearly be investigated is in motor systems, where neuronal activity leads directly to the production of a measurable output. Thus, recent studies have shown that electrical GJC plays an important role in the coordination and generation of motor outputs. In this paper we will review these new findings. Electrical GJC, which for many years has been considered a more primitive type of neuronal communication than chemical synapses, has been found to be more widespread in the brain of young than of adult mammals. This has led to the proposal that electrical coupling is less important for the production of motor activity in the adult mammalian brain [3–5]. However, recent refinements of ultrastructural techniques to demonstrate gap junctions and the use of techniques to visualize gap-junction proteins and mRNAs have demonstrated that these structures are widespread in motor areas of the adult brain. In light of these studies we will discuss the possibility that electrical GJC also plays a role in motor production in the adult mammalian nervous system.

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Electrical coupling between developing spinal motor neurons can coordinate a motor output in the absence of fast chemical synaptic transmission

It has long been known that motor neurons in the developing spinal cord are electrically coupled. This has been demonstrated in both non-limbed vertebrates [3,6,7] and mammals [8–10] (Box 1). Electrical coupling is likely to synchronize the activity of motor neurons, a role similar to that proposed for electrical GJC between neighboring cells in other areas of the CNS [11]. In a recent study, this hypothesis was tested directly by examining the motor pattern generating capability of the neonatal rat spinal cord after eliminating all action-potential (AP)-mediated synaptic transmission to motor neurons [12]. The isolated neonatal rat spinal cord contains neuronal systems, called central pattern generators or CPGs. The CPG can be activated by application of different transmitter substances [e.g. N-methyl-D-aspartate (NMDA), 5-hydroxytryptamine (5-HT) and dopamine alone or in combination] resulting in the production of a stable rhythmic motor output [13–16], closely resembling the activation of hindlimb muscles during locomotion in the intact adult rat [17] (Fig. 1a). Interestingly, when AP-mediated transmitter release is blocked either by removal of extracellular calcium or by adding tetrodotoxin (TTX) to the superfusate, application of NMDA in combination with 5-HT is still capable of producing a stable rhythmic motor output seen either as rhythmic activity (Fig. 1b) or DC oscillations (Fig. 1c) in individual ventral roots [12]. These coordinated AP-independent motor rhythms were crucially dependent on two conditions: (i) electrical coupling between and (ii) NMDA-induced oscillatory membrane properties in motor neurons. Thus, application of gap-junction blockers or removal of magnesium from the extracellular medium (blocking NMDA-induced oscillatory properties [18]) abolished the rhythms. In the case of motor neurons, the coordinated network rhythm was therefore generated by an interplay between bursting properties in individual motor neurons and the propagation of this activity through gap junctions. The strong dependency of the coordinated motor neuron oscillations on NMDA oscillatory properties differs from that described for the network oscillations observed in neurons in the inferior olive. Based on computational models, it has been proposed that coordinated oscillations in olivary neurons arise from the gap junction-mediated coupling of heterogenous or identical neurons, with no individual neuron being oscillatory on its own [19,20].

How do GJC- and NMDA-induced membrane oscillations interact? As long as a cell is coupled to other motor neurons, two rhythms can generally be observed: a relatively fast rhythm seen when the motor neuron is depolarized or at rest and an additional slow rhythm seen when the cell is hyperpolarized by current injection (Fig. 1d–f). The fast rhythm reflects the input to the neuron from the gap junction-coupled network of motor neurons, whereas the slower rhythm reflects

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Box 1. Ways of showing electrical coupling in motor neurons

The most straightforward way of showing electrical coupling between a pair of motor neurons is to perform dual recordings. At present there are no published records using this technique to study the presence of gap-junction coupling (GJC) in the intact mammalian spinal cord or brainstem. However, in acute slices combined with infrared-differential interference contrast, which allows the visualization of living neurons, paired recordings are readily performed. Taking advantage of this, Rekling *et al.* [a,b] have recently shown that abducens and hypoglossus motor neurons are strongly coupled when investigated with dual recordings.

Other techniques that have been used to demonstrate GJC between motor neurons are the antidromic collision and subthreshold stimulation tests [c–f]. Both of these tests require intracellular recording from only one motor neuron at a time. In the collision test, an orthodromic action potential (Fig. Ia1) is evoked by current injection, while the ventral root or a peripheral motor nerve containing the axons of the impaled motor neuron is stimulated to evoke an antidromic action potential (Fig. Ia2). When the time between the othrodromic and antidromic stimulation is decreased the antidromic spike collides with the outgoing orthodomic spike and fails to invade the soma and dendrites (Fig. Ia3) and eventually the initial segment. If electrical coupling is present between the impaled motor neuron and neighboring motor neurons with their axons in the stimulated nerve, the activation of the motor neurons in which the antidromic invasion is unblocked can be seen as a small depolarization (Fig. Ia4). In the subthreshold test a putative electrical coupling is seen as a small depolarization in response to low threshold antidromic stimulation of the motor axons (Fig. Ib). To exclude the possibility that the depolarization is due to recurrent chemical EPSPs, which are known to exist between motor neurons [g,h], the following tests should be performed: (i) the depolarization should be resistant to high-frequency stimulation; (ii) the size of depolarization should be insensitive to voltage; and (iii) the depolarization should persist in low or zero calcium (Fig. Ib). The final test is to try to block the electrotonic junction potential with gap-junction blockers like octanol, heptanol, carbenoxolone or halothane (Fig. Ic).

Finally, an indirect sign of electrical coupling is the passing of small dye molecules, like Lucifer yellow or neurobiotin [a,b,f]. The drawback of this method is that cells can be electrically coupled without showing dye-coupling [i].

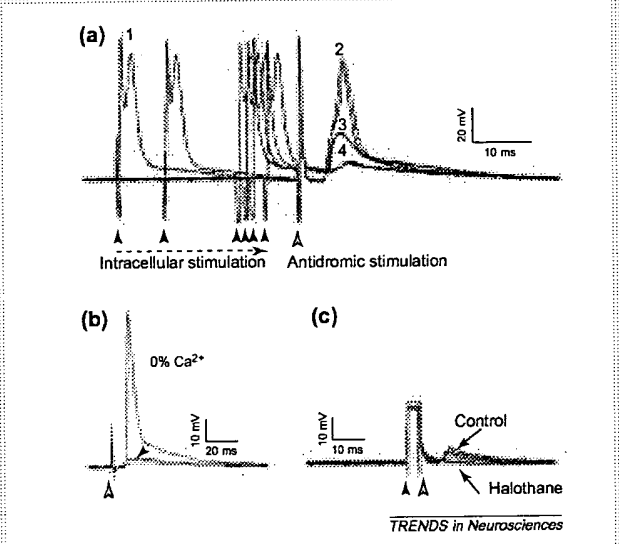

Fig. I. Criteria used to demonstrate electrical coupling among motor neurons. (a) Collisions test. Recording from a motor neuron in the isolated neonatal rat spinal cord. Action potentials (1) were elicited by current injection (closed arrowheads) during antidromic ventral-root stimulation (open arrowhead). By moving the intracellular current pulse closer to the antidromic ventral-root stimulation, the antidromic action potential (2) elicited by ventral-root stimulation failed, revealing an initial segment spike (3). When the interval between the intracellular current pulse and antidromic stimulation was decreased further, the initial segment spike also failed, revealing a putative short latency electrical coupling potential (4). Adapted from Ref. [f]. Subthreshold test and calcium insensitivity. (b) Low amplitude, short latency depolarizations in phrenic motor neuron evoked by subthreshold antidromic stimulation of the phrenic nerve in slices from mice. Subthreshold stimulation generated a graded depolarization that was calcium insensitive. Increments in the stimulation intensity eventually induced an antidromic action potential. Data from Ref. [e]. Sensitivity to gap-junction blockers. (c) Data from a newborn rat motor neuron showing that exposure of the spinal cord to halothane-saturated Ringer's solution caused successive abolishment in coupling potential amplitude until no potential could be detected. Modified from Ref. [f].

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NMDA-induced oscillations intrinsic to the neuron. This pacemaker-like activity in NMDA-induced oscillations was expressed fully when the gap junctions were blocked (Fig. 1f). The tight entrainment of the NMDA-induced oscillations in individual motor neurons by the network provides an explanation for the previous inability to change the frequency of NMDA-induced oscillations in neonatal rat and embryonic tadpole motor neurons [16,21,22] when gap junctions were still functioning.

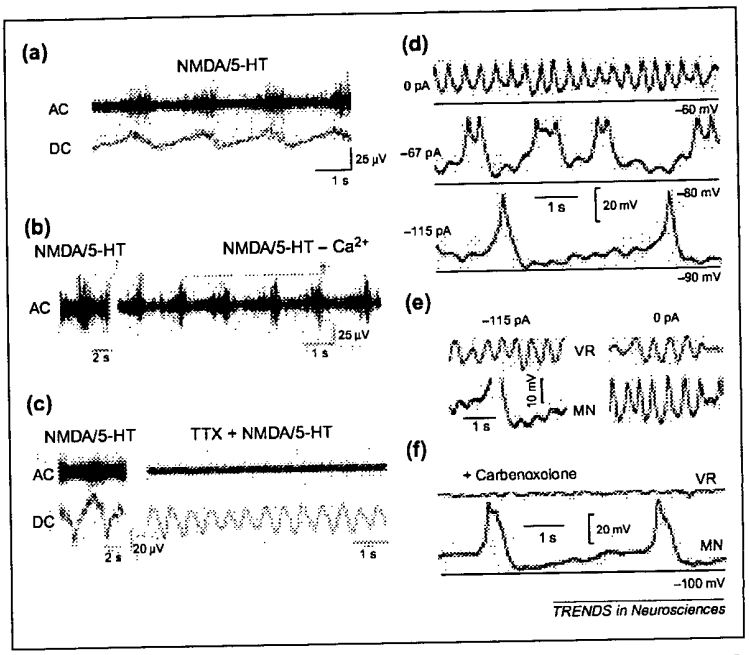

Fig. 1. Gap junction-coupled motor-neuron networks. (a) A rhythmic motor output recorded on the L5 ventral root in normal, 100% Ca^{2+} Ringer's solution evoked by a combination of NMDA (6 μM) and 5-hydroxytryptamine (5-HT) (6 μM). The top trace shows the AC recorded activity, reflecting spike activity in the root. The bottom trace shows the DC recorded activity, reflecting the attenuated membrane potential of the motor neurons with axons in the ventral root. The motor rhythms evoked by NMDA and 5-HT resemble closely those produced during natural locomotion. (b) Following removal of calcium, the rhythmic activity evoked by NMDA and 5-HT observed in normal Ringer's solution (left) could still be observed (right). (c) Following the blockade of action potentials with TTX (as reflected by the loss of AC activity in the ventral root) and, therefore, action-potential-dependent chemical synapses, NMDA and 5-HT were still able to evoke a rhythm in the DC recorded ventral root. (d) Intracellular recording from L5 motor neuron during a NMDA- and 5-HT-evoked rhythm in the presence of TTX. At hyperpolarized levels (bottom trace), both fast, small amplitude and slow, large amplitude oscillations could be observed in the neuron's membrane potential. As the neuron was depolarized, only the fast oscillation could be observed. As shown in (e), the fast oscillations (bottom traces) were coupled to the DC oscillations recorded in the ventral root (top traces). (f) The same motor neuron as in (d,e) after 20 min application of the gap-junction blocker, carbenoxolone (100 μM). The fast, low amplitude oscillations in both the motor neuron and the ventral root were abolished, but the pacemaker activity in the motor neuron persisted. Adapted from Ref. [12]. Abbreviations: 5-HT, 5-hydroxytryptamine; MN, motor neuron; NMDA, N-methyl-D-aspartate; TTX, tetrodotoxin; VR, ventral root.

Taken together, these experiments suggest that GJC between neonatal motor neurons plays a significant role in assembling activity in individual motor neurons into a coordinated motor output during rhythmic motor outputs.

Electrical coupling among motor neurons is restricted to functional groups

Several lines of evidence suggest that the electrical coupling among spinal motor neurons is relatively restricted: there is no coupling between flexor and extensor motor neurons and small or no coupling potentials between motor neurons innervating synergistic muscles [9,23]. In contrast, large electrical coupling potentials are consistently found between motor neurons innervating homonymous muscles. The gap junction and NMDA mediated oscillations described above were similarly anatomically localized. Thus, there was no coupling of the oscillations between adjacent ventral roots or between the left and the right sides of the cord [12]. This restricted anatomical extent of GJC has been also supported by dye coupling experiments in the spinal cord [10], brainstem [24] and hippocampus [11] and calcium imaging in the visual cortex [2]. Below we will consider the functional consequence of these GJC clusters.

Functional consequences of electrical coupling of motor neurons

Synchrony

An obvious consequence of electrical coupling among motor neurons is that it will tend to synchronize their firing. Synchronized firing is a widespread phenomenon in the mammalian brain [25,26], including the motor cortex [27], respiratory motor neurons [28–30] and limb motor neurons [31,32]. Several recent studies have addressed the role of GJC in synchronous motor neuron firing directly and reached conflicting results regarding the role of GJC for motor neuron synchronization. One study was performed in rhythmically active brainstem–spinal cord preparations or medullary slice preparations from young mice. In this study Bou-Flores and Berger [30] found that blocking gap junctions increases synchronous motor activity within an inspiratory burst. The investigators suggested this apparently counterintuitive observation was explained by Traub and Wong's findings [33] in hippocampal network simulations which show that introduction of electrical coupling between neurons in the networks could lead to either an increase or decrease in synchrony, depending on the strength and number of electrical synapses. In addition to the effects on synchronous activity Bou-Flores and Berger [30] also observed that blocking gap junctions reduced the inspiratory frequency and the amplitude of the motor output, suggesting a complex action of GJs in the respiratory system. Another recent study [34] has demonstrated that action potentials between motor neurons are synchronized during behavior in the developing mouse. The authors find that this synchrony disappears by the first postnatal week, at the time when GJC between motor neurons is no longer physiologically measurable with the collision test, or after administration of the gap-junction blocker, carbenoxolone. This is in contrast to the motor-neuron synchronization reported in the neonatal rat, which was not blocked by carbenoxolone [35], suggesting that other mechanisms such as chemical or ephapthic synaptic transmission could synchronize the activity of motor neurons.

However, a problem with these studies is the difficulty in interpreting the effects of gap-junction blockers. All gap-junction blockers have been reported as having side-effects on the activity of neurons in some part of the nervous system. For instance, Rekling *et al.* [24] have examined several gap-junction blockers and found that each had substantial effects on the properties of brainstem respiratory neurons. Additionally, the blockade of coupling potentials by these same substances, although

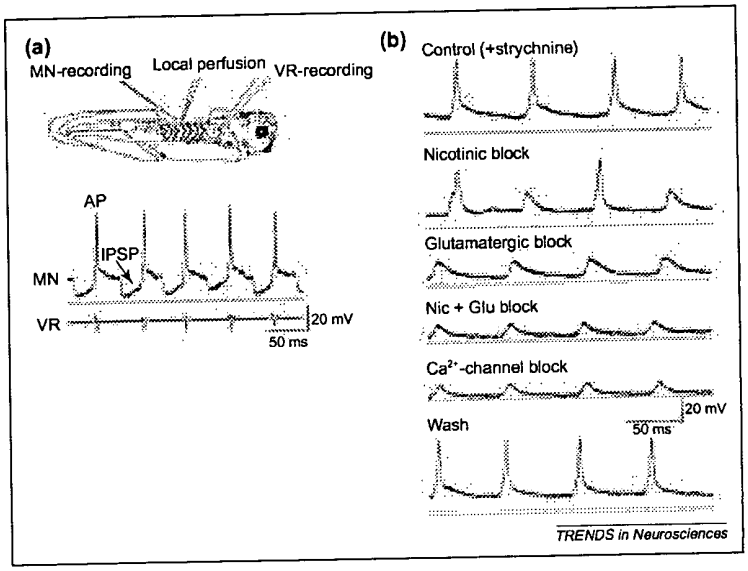

Fig. 2. Contribution of electrical coupling to rhythmic motor output in the tadpole. (a) Diagram of the tadpole preparation. Motor neuron (MN) and ventral-root (VR) recordings were performed simultaneously. A glass electrode was used for local microperfusion. Example of swimming activity recorded in a motor neuron and a ventral root. The intracellular membrane potential alternates between periods of excitation and inhibition (IPSP). (b) Components of the excitatory drive during swimming. Mid-cycle inhibition was blocked by strychnine. All other drug applications were performed by microperfusion in the area around the impaled motor neuron. Upper trace shows control recording. Second trace shows the activity when nicotinic receptors were blocked with dihydro-β-erythroidine (10 μM). Note that the action potential (AP) starts to fail in individual cycles. Blockage of glutamatergic transmission with kynurenate (2 mM) caused a large reduction in both the rhythmic depolarizations and an underlying tonic depolarization. When nicotinic (Nic) and glutamatergic (Glu) receptors were blocked or chemical synaptic transmission was blocked with cadmium, a phasic putative electrotonic coupling was revealed. Data modified from Ref. [40].

substantial, is often not complete. For these reasons, the interpretation of the effects of gap-junction blockers must clearly be performed cautiously.

Contribution to rostrocaudal excitability gradient

Gap-junction coupled motor-neuron clusters might contribute to a fundamental feature of spinal motor systems which has been observed in every vertebrate examined: the relatively stronger ability of rostral lumbar segments to produce motor rhythms as compared to caudal lumbar spinal segments [14]. This feature is usually described as a rostrocaudal gradient in excitability because rostral segments more readily produce a rhythm, and with a faster frequency than caudal segments. Despite the commonness of this rostrocaudal excitability gradient, which seems to be important for the hierarchical organization of the locomotor CPG, there is little knowledge about its cause. There is some evidence that the rostrocaudal excitability gradient might reflect local excitability differences in CPG networks in rostral and caudal segments [36]. However, the experiments with the gap junction-coordinated motor neuron-coupled network [12] suggested that such networks might also contribute to the observed gradient. Thus, the TTX-resistant oscillations evoked by NMDA/5-HT are significantly faster in rostral segments than in caudal segments [12]. The cellular mechanisms for this gradient are not known. Possible explanations are a rostrocaudal gradient in receptor sensitivity to 5-HT/NMDA and/or a rostrocaudal difference in intrinsic motor-neuron membrane properties. Another possibility is that there is a difference in the coupling strength in the gap junction-coupled motor-neuron network in rostral and caudal segments. Such effects on cycle period have been explored in an oscillating two-neuron model based on experimental work from the stomatogastric ganglion, a small motor network controlling the movement of the foregut in crustaceans [37]. This work showed that by changing the coupling strength between the neurons, the frequency of bursting could either increase or decrease, depending on the intrinsic state of the individual neurons.

Direct contribution to ongoing motor output

What is the relative contribution of motor-neuron GJC to motor-neuron excitation as compared to other mechanisms? This issue has been addressed elegantly in young *Xenopus* tadpoles, by studying the spinal locomotor system controlling swimming in these animals [38–40]. During swimming, motor neurons receive alternating excitation and inhibition that defines the active and inactive phases of the locomotor cycle. These experiments demonstrated that the excitation during swimming has three main components: (i) a glutamatergic component from premotor excitatory interneurons; (ii) a nicotinic cholinergic component via local and distant motor-neuron collaterals; and (iii) an electrotonic component from neighboring gap junction-coupled motor neurons (Fig. 2). The electrotonic coupling that comes from other phasically active motor neurons constituted about 20–25% of the total phasic excitation observed during locomotion, suggesting that these inputs provide a substantial contribution to the amplitude of the final motor drive of individual neurons. However, the authors did not attempt to block the gap junctions directly during swimming, so at the moment it is hard to evaluate whether the motor-neuron GJC also contributed to rhythm generation *per se*.

Role in activity-dependent pruning of afferent and efferent motor-neuron connections

The fact that motor-neuron coupling is mainly restricted to motor neurons innervating the same muscle or synergistic muscles has led to the notion that synchronized spike activity in homonymous motor neurons could provide a Hebbian mechanism for the establishment of afferent and efferent motor-neuron synapses [2–5]. For example, synchronized activity in homonymous motor neurons will lead to synchronous activity in the target muscles, which in turn leads to synchronous activity in proprioceptive afferents projecting to the same homonymous motor neurons. This coordinated activity in proprioceptors coming from the same

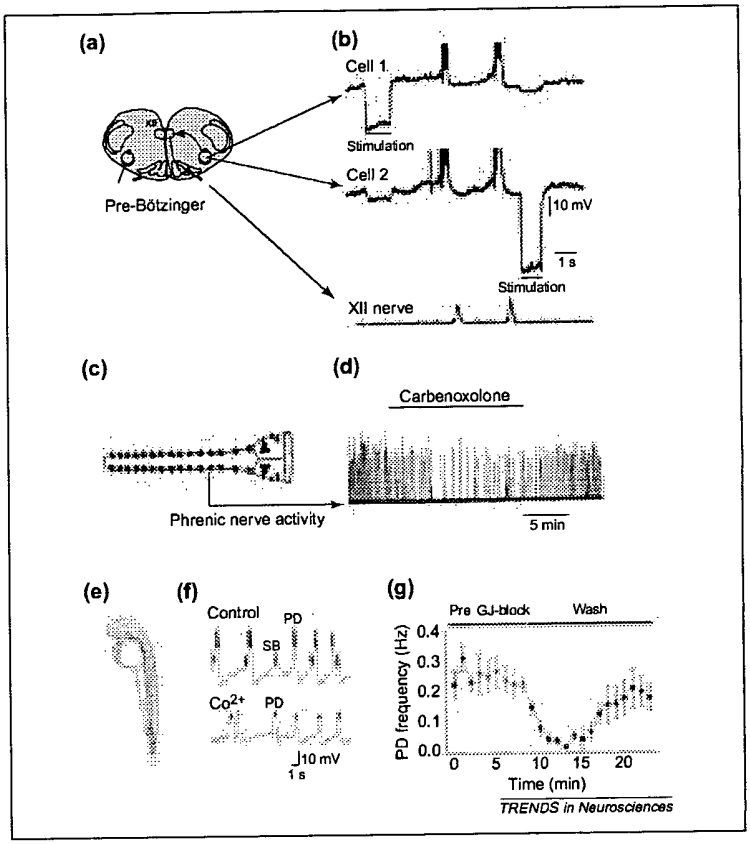

Fig. 3. Electrical coupling in premotor-neuron networks. Type-1 respiratory neurons are bidirectionally electrically coupled (a,b). (a) Schematic diagram of medullary slice preparation containing the pre-Bötzinger complex. Respiratory activity is recorded in the XII nerve [lower trace in (b)] and intracellularly from type-1 inspiratory neurons. Adapted from Ref. [69]. (b) Simultaneous recordings from two type-1 neurons. A hyperpolarizing pulse (bars below intracellular traces) in either neuron elicited an attenuated hyperpolarizing response in the other electrically coupled neuron. Adapted from Ref. [24]. Gap-junction blocking reduces the respiratory frequency in newborn mice (c-d). (c) Schematic diagram of brain stem–spinal cord preparation. The respiratory activity is recorded in the C_4 nerve. Adapted from Ref. [69]. (d) Carbenoxolone (100 μM) was added to the Ringer's solution superfusing the preparation to block gap junctions. Adapted from Ref. [30]. Electrical coupling is responsible for spontaneous periodic depolarizations in the zebrafish embryo (e–g). (e) Schematic of a 25 h zebrafish embryo. (f) Recordings from a motor neuron, showing the spontaneous periodic depolarizations (PD) and synaptic bursts (SB). Cobalt (Co^{2+}, 2 mM) blocked synaptic bursts but not periodic depolarizations. (g) Acidification with NH_4Cl, which uncouples gap junctions (GJ) blocks the periodic depolarizing (average data of six cells). Modified from Ref. [7].

muscle would be strengthened through activity-dependent mechanisms, whereas the non-synchronized inputs are eliminated [2,3]. Although evidence suggests that such activity-dependent mechanisms are not crucial in establishing the specificity of direct Ia proprioceptive feedback to motor neurons [41], these mechanisms might be involved in the specification of other synapses to motor neurons, such as those from interneuronal or descending systems. Similarly, synchronization of motor-neuron activity has been proposed to mediate activity-dependent pruning of the polysynaptic innervation of mammalian muscles that takes place during the first postnatal weeks [4,5,10]. According to this proposal, synchronized activity in homonymous motor neurons during embryonic and early postnatal life promotes the innervation of individual muscle fibers by multiple motor axons. The transition from this polysynaptic innervation pattern to the mature pattern of single innervation by the end of the first postnatal week has been suggested to result from a reduction in synchronized activity among motor neurons, leading to a competitive process of synapse elimination. This reduction in motor-neuron synchronization was suggested to result from the gradual loss of GJC between motor neurons taking place over this same period. Consistent with these hypotheses, Personius and Balice-Gordon [34] demonstrated that synchronization among motor neurons is present during the first postnatal week but disappears by the end of the second week. These results suggest that GJC among motor neurons is transient and mainly serves a developmental function. We will discuss this issue further below.

Electrical coupling between pre-motor interneurons

Gap junctions among interneurons play an important role for the function of neural networks generating rhythmic motor outputs in invertebrates [42,43]. Few studies have addressed this issue in vertebrate motor systems. Perhaps one reason for this is that it is technically more difficult to demonstrate electrical coupling between interneurons because it most often requires paired recordings.

Using paired recordings, Rekling *et al.* [24] demonstrated bi-directional electrical coupling between rhythmogenic type-1 neurons in the pre-Bötzinger complex – the hypothesized site for respiratory rhythm generation [44] – in neonatal mice (Fig. 3a,b). Bi-directional electrical coupling could act in combination with intrinsic membrane properties to synchronize and augment oscillatory respiratory rhythms. As respiratory motor neurons are also strongly coupled in neonates [24,45–47], widespread synchronization seems to be present in the respiratory system, perhaps a reflection of the fact that many respiratory muscles are activated in concert. Bou-Flores and Berger [30] (Fig. 3c,d) found that blocking gap junctions, in addition to increasing motor-neuron synchronization within the burst, consistently reduced the respiratory frequency. The substrate for this latter effect is likely to be in the pre-Bötzinger complex that controls the frequency of the respiratory rhythm and could involve a reduction in the mutual re-excitation of rhythmogenic type-1 neurons, thereby decreasing the excitability level in the pre-Bötzinger complex [24]. Modulation of GJC might therefore be a means of changing the respiratory cycle frequency. Moreover, modeling studies have shown that synchronized inputs will increase the force output from motor neurons more than asynchronous inputs for a given input rate [27]. Changes in GJC in rhythm generating networks are therefore likely also to affect the force output.

The power of electrical coupling in premotor network is perhaps most clearly illustrated by recent findings in the zebrafish embryo [7]. In these embryos, aged 19–24 h, periodic spontaneous bursts can be recorded in motor neurons. These bursts are blocked by tetrodotoxin and cadmium but are insensitive to blocking of chemical synaptic transmission by cobalt (Fig. 3f), specific glutametergic receptor blockers, and γ-aminobutyric acid (GABA) or glycine receptor blockers. In contrast, the periodic spontaneous bursting is abolished by treatments that uncouple gap junctions (Fig. 3g). These experiments suggest that in embryonic life rhythm generation can function in the absence of chemical synaptic transmission and that the premotor neurons at this stage might be directly coupled through gap junctions to motor neurons.

Electrical coupling has also been described between descending fibers and spinal interneurons in both the lamprey [48] and the goldfish [49]. This arrangement is similar to that seen in the crayfish where electrical connections are observed between giant fibers and tail motor neurons [50]. The role of such connections in vertebrate motor control has not yet been evaluated.

Electrical coupling in adult mammalian motor systems

In the above description we have concentrated on data from pre- and early postnatal animals. Several studies have indicated that electrical GJC, although present in spinal cords from adult aquatic vertebrates (frog [51], lamprey [48] and goldfish [49]), are transient in nature in the mammalian spinal cord [3–5]. This has led to the notion that electrical gap junctions might not play any role in coordinating motor acts in adult mammals. Below we will review evidence which suggests that this view might need to be revised.

Anatomical evidence for GJC in the adult mammalian spinal cord

Indirect evidence for electrical GJC comes from molecular and anatomical studies. Gap-junction channels are composed of hexamers of connexin proteins. In rodents about 15 connexin genes have currently been characterized [52]. Using *in situ* hybridization and immunostaining, Chang *et al.* [10] found that immature spinal motor neurons express five connexins, Cx36, Cx37, Cx 40, Cx43 and Cx45. Although a down regulation of Cx40 and Cx45 is observed in adult animals as compared to neonatal rats, the expression of the three other connexins remains unchanged. Rash and colleagues [53] confirmed the presence of Cx36 and Cx43 in the spinal cord of adult rats. They also showed, however, using freeze-fracture replica immunogold labeling, that Cx36 is found in interneurons and motor neurons, whereas Cx43 was detected in astrocytes and ependomocytes. This result is in agreement with investigations from other areas of the CNS where Cx36 appears almost exclusively in neurons [54]. A recent study in the adult mouse brain has revealed expression of an additional connexin gene Cx47 that is transcribed in spinal motor neurons and interneurons [55]. The electrical conductance of this channel was found to be threefold higher than the Cx36. Evidence for a widespread distribution of GJC was given by Rash *et al.* [56] using 'grid-mapped-freeze-fracture', which allows researchers to identify tiny structures. With this technique it was shown that mixed synapses, consisting of both chemical and electrical synapses, were present in all areas of the adult spinal cord. In summary, these experiments strongly suggest that the adult mammalian spinal cord contains the substrate for electrical communication between cells. However, a demonstration of whether this substrate is in fact capable of mediating electrical transmission requires physiological experiments.

Physiological evidence for electrical coupling in adult motor systems

At present only a few studies have addressed whether these anatomical substrates mediate electrical coupling between motor neurons in the adult. Using the collision and subthreshold stimulation tests (Box 1), three studies have demonstrated that adult cat brainstem and spinal motor neurons receive short-latency depolarizations from other motor neurons [57–59]. These depolarizations were interpreted as electrotonic coupling rather than recurrent excitation [60] because they obeyed the classical criteria for coupling potentials (Box 1). Dye coupling has also been described in adult rat ambiguous motor neurons [61]. In contrast to these studies a recent study by Chang *et al.* [62] was not able to find dye coupling or signs of electrical coupling in adult cat motor neurons unless the motor neurons had undergone an axotomy 3–4 weeks in advance. At present there is no obvious explanation for these conflicting results. It shows, however, that more studies are needed. Since GJC is modifiable by intracellular calcium and pH, by neuromodulators, and by synaptic activity [63–65] it is likely that the state of the preparation at the time of recording will affect the ability to detect electrical coupling. A dendritic location of gap junctions in motor neurons could also reduce the size of the signal to a level that is not measured with collision tests. Because of these concerns, a clear evaluation of the potential existence of GJC in the adult mammalian motor neurons will require dual intracellular recordings. In other areas of the mammalian brain where this technique is used routinely, electrical coupling has been found to be much more abundant than previously believed [66–68]. It might turn out that this ancient type of intercellular signaling also has a role in adult motor behavior, for example by synchronizing motor-neuron firing during normal and involuntary (Box 2) movements.

Box 2. Fasciculations

Fasciculations are involuntary twitches in small portions of a muscle caused by synchronous discharge in several motor units. Fasciculations can be a first sign of a motor-neuron disease such as amyotrophic lateral sclerosis. However most fasciculations are benign, and are experienced by healthy people. Among other factors, benign fasciculations are precipitated by fatigue, stress, caffeine and smoking. One possible cause among many for these benign fasciculations is a temporary increase in the strength of gap-junction coupling amongst motor neurons, leading to the simultaneous contractions in several motor units.

Conclusions

As detailed in this review, there have been several recent studies examining the role of gap junctions in motor systems. These studies have demonstrated the presence of gap junctions between neurons at many levels of the motor system, in both motor neurons and in premotor pattern generating circuits. Although gap junctions are clearly prevalent in early development, there is also considerable evidence that, at least anatomically, the substrates for gap junctions are present in adults as well. Further, GJC has been shown to be capable of bringing about robust coordination patterns, even in the absence of chemical synapses, and has been shown to mediate synchronization of neurons during motor behaviors. Despite the increased understanding these studies have provided about the presence and function of gap junctions, a full understanding of the role of gap junctions in motor function remains unclear. How the distinct integrative functions of electrical and chemical synapses are utilized to allow for the complex information processing taking place in neural systems requires considerable further research. Whatever the precise role of gap junctions in the production of behavior, the studies reviewed here, demonstrating the prevalence of gap junctions and their powerful physiological consequences, suggest that this role will be substantial.

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WEST Search History

DATE: Saturday, September 28, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT; PLUR=YES; OP=ADJ			
L8	L5 and gap junction	3	L8
L7	L3 and ion channel	12	L7
L6	L3 and gap junction	11	L6
L5	L2 and ion channel	244	L5
L4	L2 and gap junction	31	L4
L3	L1 and cardiomyocyte	149	L3
L2	L1 and neuron$	2897	L2
L1	transplant$	25308	L1

9/28/02 AM

END OF SEARCH HISTORY

AMJ

20/3,AB/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13513142 BIOSIS NO.: 200200141963

Gap junctions and motor behavior.

AUTHOR: Kiehn Ole(a); Tresch Matthew C
AUTHOR ADDRESS: (a)Dept of Neuroscience, Karolinska Institutet, Retzius Vag 8, S-17177, Stockholm**Sweden E-Mail: Ole.Kiehn@neuro.ki.se

JOURNAL: Trends in Neurosciences 25 (2):p108-115 February, 2002
MEDIUM: print
ISSN: 0166-2236
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The production of any motor behavior requires coordinated activity in motor neurons and premotor networks. In vertebrates, this coordination is often assumed to take place through chemical synapses. Here we **review** recent data suggesting that electrical **gap - junction** coupling plays an important role in coordinating and generating motor outputs in embryonic and early postnatal life. Considering the recent demonstration of a prevalent expression of **gap - junction** proteins and **gap - junction** structures in the adult mammalian spinal cord, we suggest that neuronal **gap - junction** coupling might also contribute to the production of motor behavior in adult mammals.

2002
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13832377 BIOSIS NO.: 200200461198

Structural and functional diversity of connexin genes in the mouse and human genome.

AUTHOR: Willecke Klaus(a); Eiberger Juergen; Degen Joachim; Eckardt Dominik ; Romualdi Alessandro; Gueldenagel Martin; Deutsch Urban; Soehl Goran(a)
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JOURNAL: Biological Chemistry 383 (5):p725-737 May, 2002
MEDIUM: print
ISSN: 1431-6730
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Gap junctions are clustered channels between contacting cells through which direct intercellular communication via diffusion of ions and metabolites can occur. Two hemichannels, each built up of six connexin protein subunits in the plasma membrane of adjacent cells, can dock to each other to form conduits between cells. We have recently screened mouse and human genomic data bases and have found 19 connexin (Cx) genes in the mouse genome and 20 connexin genes in the human genome. One mouse connexin gene and two human connexin genes do not appear to have orthologs in the other genome. With three exceptions, the characterized connexin genes comprise two exons whereby the complete reading frame is located on the second exon. Targeted ablation of eleven mouse connexin genes revealed basic insights into the functional diversity of the connexin gene family. In addition, the phenotypes of human genetic disorders caused by mutated connexin genes further complement our understanding of connexin functions in the human organism. In this **review** we compare currently identified connexin genes in both the mouse and human genome and discuss the functions of gap junctions deduced from targeted mouse mutants and human genetic disorders.

2002

20/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13808278 BIOSIS NO.: 200200437099
Gap junctions: Structure and function (Review).
AUTHOR: Evans W Howard(a); Martin Patricia E M
AUTHOR ADDRESS: (a)Departments of Medical Biochemistry and Diagnostic Radiology, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN**UK E-Mail: wmbwhe@cardiff.ac.uk
JOURNAL: Molecular Membrane Biology 19 (2):p121-136 April-June, 2002
MEDIUM: print
ISSN: 0968-7688
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Gap junctions are plasma membrane spatial microdomains constructed of assemblies of channel proteins called connexins in vertebrates and innexins in invertebrates. The channels provide direct intercellular communication pathways allowing rapid exchange of ions and metabolites up to apprx1 kD in size. Approximately 20 connexins are identified in the human or mouse genome, and orthologues are increasingly characterized in other vertebrates. Most cell types express multiple connexin isoforms, making likely the construction of a spectrum of heteromeric hemichannels and heterotypic gap junctions that could provide a structural basis for the charge and size selectivity of these intercellular channels. The precise nature of the potential signalling information traversing junctions in physiologically defined situations remains elusive, but extensive progress has been made in elucidating how connexins are assembled into gap junctions. Also, participation of **gap junction** hemichannels in the propagation of calcium waves via an extracellular purinergic pathway is emerging. Connexin mutations have been identified in a number of genetically inherited channel communication-opathies. These are detected in connexin 32 in Charcot Marie Tooth-X linked disease, in connexins 26 and 30 in deafness and skin diseases, and in connexins 46 and 50 in hereditary cataracts. Biochemical approaches indicate that many of the mutated connexins are mistargeted to gap junctions and/or fail to oligomerize correctly into hemichannels. Genetic ablation approaches are helping to map out a connexin code and point to specific connexins being required for cell growth and differentiation as well as underwriting basic intercellular communication.

2002

20/3,AB/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13619936 BIOSIS NO.: 200200248757

Gap junctions and tumour progression.

AUTHOR: Naus Christian C G(a)
AUTHOR ADDRESS: (a)Department of Anatomy and Cell Biology, The University of Western Ontario, London, ON, N6A 5C1**Canada E-Mail: cnaus@uwo.ca

JOURNAL: Canadian Journal of Physiology and Pharmacology 80 (2):p136-141 February, 2002
MEDIUM: print
ISSN: 0008-4212
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Gap junctional intercellular communication has been implicated in growth control and differentiation. The mechanisms by which connexins, the **gap junction** proteins, act as tumor suppressors are unclear. In this **review**, several different mechanisms are considered. Since transformation results in a loss of the differentiated state, one mechanism by which gap junctions may control tumour progression is to promote or enhance differentiation. Processes of differentiation and growth control are mediated at the genetic level. Thus, an alternative or complimentary mechanism of tumour suppression could involve the regulation of gene expression by connexins and gap junctional coupling. Finally, **gap junction** channels from a conduit between cells for the exchange of ions, second messengers, and small metabolites. It is clear that the sharing of these molecules can be rather selective and may be involved in growth control processes. In this **review**, examples will be discussed that provide evidence for each of these mechanisms. Taken together, these findings point to a variety of mechanisms by which connexins and the **gap junction** channels that they form may control tumour progression.

2002

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Set	Items	Description
S1	96549	ION(W)CHANNEL
S2	863	S1 AND (TRANSPLANT? OR IMPLANT?)
S3	698	RD (unique items)
S4	16	S3 AND EPILEPSY
S5	1	S3 AND FETAL(W)NEURON?
S6	1	S3 AND TRANSPLANT?(W)NEURON?
S7	6	S3 AND GAP(W)JUNCTION
S8	6	S3 AND CARDIOMYOCYTE?
S9	2009250	TRANSPLANT?
S10	30836	S9 AND NEURON?
S11	8679	S10 AND TRANSPLANT?/TI
S12	3141	S11 AND NEURON?/TI
S13	2892	S12 NOT PY>2000
S14	0	S13 AND ION(W)CHANNEL
S15	0	S13 AND GAP(W)JUNCTION
S16	19	S13 AND TRANSPORTER
S17	7	RD (unique items)
S18	2921	NEURON? AND GAP(W)JUNCTION
S19	2435	S18 NOT PY>2000

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Dialog
file: medicine
Amz
9/28/02

19/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13122396 BIOSIS NO.: 200100329545
Components of astrocytic intercellular calcium signaling.
AUTHOR: Scemes Eliana(a)
AUTHOR ADDRESS: (a)Department of Neuroscience, Kennedy Center, Albert Einstein College of Medicine, 1410 Pelham Parkway South, Rm 924, Bronx, NY, 10461**USA
JOURNAL: Molecular Neurobiology 22 (1-3):p167-179 August-October-December, 2000
MEDIUM: print
ISSN: 0893-7648
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: It has become evident that astrocytes play major roles in central nervous system (CNS) function. Because they are endowed with ion channels, transport pathways, and enzymatic intermediates optimized for ionic uptake, degradation of metabolic products, and inactivation of numerous substances, they are able to sense and correct for changes in neural microenvironment. Besides this housekeeping role, astrocytes modulate **neuronal** activity either by direct communication through gap junctions or through the release of neurotransmitters and/or nucleotides affecting nearby receptors. One prominent mode by which astrocytes regulate their own activity and influence **neuronal** behavior is via Ca2+ signals, which may be restricted within one cell or be transmitted throughout the interconnected syncytium through the propagation of intercellular calcium waves. This review aims to outline the most recent advances regarding the active communication of astrocytes that is encoded by intracellular calcium variation.

19/3,AB/7 (Item 7 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12965055 BIOSIS NO.: 200100172204
2,3,7,8-tetrachlorodibenzo-p-dioxin alters hippocampal astroglia- neuronal **gap junctional communication.**
AUTHOR: Legare M E(a); Hanneman W H; Barhoumi R; Burghardt R C; Tiffany-Castiglioni E
AUTHOR ADDRESS: (a)Department of Environmental Health, School of Veterinary Medicine, Colorado State University, Ft. Collins, CO, 80523: mlegare@cvmbs.colostate.edu**USA
JOURNAL: Neurotoxicology (Little Rock) 21 (6):p1109-1116 December, 2000
MEDIUM: print
ISSN: 0161-813X
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Halogenated aromatic hydrocarbons (HAHs) such as dibenzo-p-dioxins are known to alter cognitive function. However, the cellular basis of this disruption is not well understood. One possible deleterious effect of exposure to HAHs could be on gap junctional intercellular communication (GJIC) between **neurons** and astroglia in the brain. As such, this study examined the effects of the highly toxic prototypic HAH, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on GJIC in rat hippocampal primary cell culture. Initial measurements of fluorescence recovery after photobleaching (gap-FRAP) showed dye transfer between astroglia and **neurons** . N-octanol, a lipophilic alcohol known to uncouple cells by decreasing the open probability of gap junctional channels blocked astroglial- **neuronal** (A-N) communication as well as astroglial-astroglial (A-A) communication. TCDD initially downregulated GJIC between **neurons** and astroglia of treatment, but had no effect on astroglial cell pairs. These results indicate the presence of GJIC between **neurons** and astroglia in culture and demonstrate different sensitivities of **gap junction** responses to TCDD in homologous and heterologous cell pairs. The finding that 2,3,7,8-TCDD disrupts GJIC through A-N but not A-A channels may have important implications for impaired brain function resulting from developmental exposure to TCDD.

2000

19/3,AB/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12927432 BIOSIS NO.: 200100134581
Gap junctions contribute to the propagation of acetylcholine-mediated activity in the hypothalamus in vitro.
AUTHOR: Li B(a); Denisova J V; Belousov A B
AUTHOR ADDRESS: (a)Tulane University, New Orleans, LA**USA
JOURNAL: Society for Neuroscience Abstracts 26 (1-2):pAbstract No-8044 2000
MEDIUM: print
CONFERENCE/MEETING: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000
SPONSOR: Society for Neuroscience
ISSN: 0190-5295
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Acetylcholine (ACh) appears to be the major excitatory neurotransmitter in some regions of the CNS (e.g. retina and spinal cord) during early stages of development, before glutamate begins to play this role at the later states. At early stages, gap junctions (electrical synapses) are also expressed in these CNS regions and act in combination with the cholinergic synaptic drive to produce spontaneous excitatory activity (Catsicas et al. 1998; Milner and Landmesser 1999). Our data demonstrate (Belousov et al., 2000) that during a chronic (6-14 days) blockade of glutamate neurotransmission (with AP5 100 muM and CNQX 10 muM) in rat hypothalamic cultures, ACh, a neurotransmitter normally exhibiting only weak activity in the hypothalamus, becomes the major excitatory neurotransmitter and supports the excitation/inhibition balance in these cultures. We tested whether gap junctions contribute to the propagation of excitatory ACh activity in hypothalamic cultures following glutamate receptor blockade. **Gap junction** blocker carbenoxolone (5-100 muM) reduced ACh-mediated intracellular Ca2+ raises in **neurons** on a dose-dependent manner. Labeling of single **neurons** with neurobiotin in the patch-electrode medium revealed the spreading of fluorescent dye between cells. Additionally, immunostaining of cultures for a **gap junction** protein connexin-32 revealed the expression of this protein in cultured **neurons** and glial cells. Our data suggest that electrical synapses contribute to the propagation of ACh excitation following a decrease in glutamate transmission. These data also support the possibility for developmental aspects in glutamate/ACh/ **gap junction** interactions in the hypothalamus in vitro following a decrease in glutamate excitation.
2000

19/3,AB/10 (Item 10 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12927214 BIOSIS NO.: 200100134363
Connexin expression in identified inhibitory neurons **of somatosensory cortex.**
AUTHOR: Keller A(a); Thompson A J; Shipley M T; Priest C A
AUTHOR ADDRESS: (a)University of Maryland School of Medicine, Baltimore, MD **USA
JOURNAL: Society for Neuroscience Abstracts 26 (1-2):pAbstract No-7745 2000
MEDIUM: print
CONFERENCE/MEETING: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000
SPONSOR: Society for Neuroscience
ISSN: 0190-5295
RECORD TYPE: Abstract

LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Recent studies indicate that electrical coupling among cortical **neurons** is significantly more extensive than previously thought. Specifically, recordings in cortical slices from young rats reveal the existence of electrically-coupled networks of cortical **neurons** involving parvalbumin (PV) or somatostatin (SOM) containing GABAergic **neurons** (Nature 402:72 & 402:75). Our aims were to determine the type(s) of connexin proteins (Cx) expressed by PV and SOM **neurons** , and whether these proteins are expressed in cortical **neurons** , in adult mice, in vivo.With the use of double-labeling immunofluorescence we demonstrate that Cx 32 and Cx 43 are expressed in cortical **neurons** labeled with antisera against the **neuronal** markers **neuronal** nuclei (NeuN) and neurofilament 145. Cx 43, but not Cx 32, also co-localized with glial fibrillary acidic protein (GFAP)-containing cells. SOM and PV-containing **neurons** in both supra- and infragranular layers were immuno-positive for Cx 32 and Cx 43. Cx 32 preferentially labeled the somata of SOM and PV **neurons** , whereas Cx 43 was localized primarily in their dendrites. These findings demonstrate that SOM and PV-containing **neurons** in the adult somatosensory cortex express **gap - junction** proteins. This suggests that electrical coupling among these inhibitory **neurons** may persist into adulthood, and that these interactions may be an important substrate for **neuronal** communication.

2000
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8/3,AB/2 (Item 1 from file: 94)
DIALOG(R)File 94:JICST-EPlus

05018670 JICST ACCESSION NUMBER: 01A0973811 FILE SEGMENT: JICST-E

Molecular mechanism of cardiac hypertrophy and cardiac development.

KOMURO I (1)
(1) Chiba Univ., Chiba

Chiba Igaku Zasshi(Chiba Medical Journal), 2001, VOL.77,NO.5, PAGE.301-311
, REF.55
JOURNAL NUMBER: G0640ABD ISSN NO: 0303-5476 CODEN: CIZAA
UNIVERSAL DECIMAL CLASSIFICATION: 591.112.2.05+591.412 616.11/.16
LANGUAGE: English COUNTRY OF PUBLICATION: Japan
DOCUMENT TYPE: Journal
ARTICLE TYPE: Review article
MEDIA TYPE: Printed Publication

ABSTRACT: To understand the heart failure, it is important to elucidate the mechanism of the development of cardiac hypertrophy. Hemodynamic overload, namely mechanical stress, is a major cause for cardiac hypertrophy. To dissect the signaling pathways from mechanical stress to cardiac hypertrophy, we developed the in vitro device by which mechanical stress can be imposed on cardiac myocytes of neonatal rats cultured in the serum-free condition. Passive stretch of cardiac myocytes cultured on silicone membranes induced various hypertrophic responses such as activation of phosphorylation cascades of many protein kinases, expression of specific genes and an increase in protein synthesis. During this process, secretion and production of vasoactive peptides such as angiotensin II and endothelin-1, are increased and they played critical roles in the induction of these hypertrophic responses. Recently candidates for the "mechanoreceptor" which receive mechanical stress and convert it into intracellular biochemical signals have been demonstrated. To treat heart failure, gene therapy and cell **transplantation** are hopeful strategies. To enable these novel treatments, it is important to understand how normal cardiac myocytes are differentiated. We have isolated a key gene which plays a critical role in cardiac development. A cardiac homeobox-containing gene Csx is expressed in the heart and the heart progenitor cells from the very early developmental stage, and targeted disruption of the murine Csx results in embryonic lethality due to the abnormal looping morphogenesis of the primary heart tube. With a cardiac zinc finger protein GATA4, Csx induces **cardiomyocyte** differentiation of teratocarcinoma cells as well as upregulation of cardiac genes. Mutations of human Csx cause various congenital heart diseases including atrial septal defect, ventricular septal defect, tricuspid valve abnormalities and atrioventricular block. (author abst.)

17/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12619269 BIOSIS NO.: 200000372771
Real-time dynamics of dopamine released from neuronal transplants **in experimental Parkinson's disease.**
AUTHOR: Cragg S J(a); Clarke D J; Greenfield S A(a)
AUTHOR ADDRESS: (a)Department of Pharmacology, University of Oxford, Oxford, England, OX1 3QT**UK
JOURNAL: Experimental Neurology 164 (1):p145-153 July, 2000
MEDIUM: print
ISSN: 0014-4886
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Intrastriatal **transplantation** of foetal midbrain dopamine (DA) **neurons** ameliorates the fundamental symptoms of dopaminergic denervation in clinical and experimental parkinsonism despite providing only restricted reinnervation. To understand how DA function is restored by these grafts we used fast-scan cyclic voltammetry at a carbon-fiber microelectrode in vitro to monitor directly and in "real time" the dynamics of graft-derived DA. Simulations of Michaelis-Menten kinetics were used to model the experimental observations. We show that the concentration of DA released by a single depolarizing pulse is significantly lower in grafted than intact striata. On the other hand, the extracellular lifetime of DA in grafts is extended due to a marked reduction in the rate maximum (Vmax) for DA reuptake by the DA **transporter** . Moreover, variations in Vmax and release occur in parallel: where DA release is lowest, Vmax is lowest and vice versa. The consequences of these dynamics are twofold. First, during repeated depolarization at a physiological firing frequency, when net extracellular concentrations reflect DA release versus uptake, ambient levels of extracellular DA within the graft are restored to normal. Second, the protracted extracellular lifetime of DA will increase the number and extracellular sphere of its postsynaptic actions. This effect will be most prominent where DA availability (and thus Vmax) is most restricted. Thus, these data demonstrate that dopaminergic grafts restore striatal dopaminergic function with extracellular dynamics of DA that are different from those of intact striatum but which can normalize ambient DA levels and permit transmission over an extended sphere.

2000

17/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12589373 BIOSIS NO.: 200000342875
Development of glycine-accumulating neurons **in retinal** transplants .
AUTHOR: Sharma Rajesh Kumar(a)
AUTHOR ADDRESS: (a)Department of Ophthalmology, University Hospital of Lund, S-221 85, Lund**Sweden
JOURNAL: Ophthalmologica 214 (4):p264-270 July-August, 2000
MEDIUM: print
ISSN: 0030-3755
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Previous studies have shown that the fetal retina not only survives **transplantation** but also continues to develop and differentiate in the host eye. Several structural and functional proteins

have been demonstrated in the **transplanted** retinas, and the presence of such proteins has been taken as evidence for the capability of retinal **transplants** to function. Glycine is an important inhibitory neurotransmitter and is found in a large number of the retinal **neurons** . Uptake of glycine rather than de novo synthesis is the main source of glycine in glycinergic **neurons** . The present study examined whether glycine-accumulating **neurons** develop normally in rabbit retina **transplants** . Embryonic day (E) 15 rabbit retinas were **transplanted** into the eyes of adult rabbits of the same strain. **Transplants** were allowed to survive for various times so that the grafts attained the equivalent ages of (donor age + survival time) E 19, 22 and 29 and postnatal days (PN) 2, 5, 9, 12, 19 and 58. On formaldehyde-fixed cryostat sections of these **transplants** , glycine-accumulating **neurons** were demonstrated by immunohistochemistry by using an antibody against one of the glycine transporters: GLYT1. Immunoreactivity was first detected 2 days before birth and increased with age until it reached its mature level at PN 19. The immunoreactivity was found in cells belonging to the inner retinal layers, and in plexiform layers of the **transplant** equivalent to the normal inner and the outer plexiform layers. In places these cells integrated well with similar cells in the host. In the host retina, the immunoreactivity was found in proximal cell layers of the inner nuclear layer, in certain bipolar cells, and in the inner and the outer plexiform layers. The immunoreactivity was preserved even in the degenerated retina overlying the retinal graft. In conclusion, the present study demonstrates that glycine-accumulating **neurons** develop, integrate and survive in retinal **transplants** .
2000

17/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10421744 BIOSIS NO.: 199699042889
Characterization and transplantation **of two** neuronal **cell lines with dopaminergic properties.**
AUTHOR: Adams Frank S; La Rosa Francisco G; Kumar Sanjay; Edwards-Prasad Judith; Kentroti Susan; Vernadakis Antonia; Freed Curt R; Prasad Kedar N
AUTHOR ADDRESS: Center Vitamins Cancer Res., Dep. Radiol., Univ. Colorado Health Sci. Cent., Box C278, 4200 E. Ninth**USA
JOURNAL: Neurochemical Research 21 (5):p619-627 1996
ISSN: 0364-3190
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Immortalized rat mesencephalic cells (1RB-3AN-27) produced dopamine (DA) at a level that was higher than produced by undifferentiated or differentiated murine neuroblastoma cells (NBP-2) in culture. Treatment of 1RB-3AN-27, and NBP-2 cells with a cAMP stimulating agent increased tyrosine hydroxylase (TH) activity and the intensity of immunostaining for the DA **transporter** protein (DAT). 1RB-3AN-27 cells were labelled with primary antibodies to **neuron** specific enolase (NSE) and nesting and exhibited very little or no labeling with anti-glial fibrillary acidic protein (GFAP). 1RB-3AN-27 cells exhibited beta- and alpha-adrenoreceptors, and prostaglandin E-1 receptors, all of which were linked to adenylate cyclase (AC). Dopamine receptor (D-1) and cholinergic muscarinic receptors linked to AC were not detectable. The levels of PKC-alpha and PKC-beta isoforms were higher than those of PKC-gamma and PKC-delta in 1RB-3AN-27 cells. The 1RB-3AN-27 cells were more effective in reducing the rate of methamphetamine-induced turning in rats with unilateral 6-OHDA lesion of the nigrostriatal system than differentiated NBP-2 cells. The grafted 1RB-3AN-27 were viable as determined by DiI labelling, but they did not divide and did not produce T-antigen protein; however, when these grafted cells were cultured in vitro, they resumed production of T-antigen and proliferated after the primary glia cells and **neurons** of host brain died due to maturation and subsequent

degeneration. Examination of H&E stained sections of the grafted sites revealed no evidence of infiltration of inflammatory cells in the grafted area suggesting that these cells were not immunogenic. They also did not form tumors.

1996

17/3,AB/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05918273 Genuine Article#: XG251 Number of References: 61
Title: Glial and endothelial cell response to a fetal transplant **of purified** neurons (ABSTRACT AVAILABLE)
Author(s): RostaingRigattieri S; FloresGuevara R; Peschanski M; Cadusseau J (REPRINT)
Corporate Source: FAC MED,INSERM, UNITE 421, IM3, 9 RUE GEN SARRAIL/F-94010 CRETEIL//FRANCE/ (REPRINT); FAC MED,INSERM, UNITE 421, IM3/F-94010 CRETEIL//FRANCE/
Journal: NEUROSCIENCE, 1997, V79, N3 (AUG), P723-734
ISSN: 0306-4522 Publication date: 19970800
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB
Language: English Document Type: ARTICLE
Abstract: Astrocytes, microglia and endothelial cells display very specific phenotypic characteristics in the intact adult CNS, which appear quite versatile when grown in culture without **neurons** . Indirect evidence from in vitro co-culture studies and analysis of the effects of specific **neuronal** removal in vivo, does accordingly favour a role of **neurons** for the phenotypic repression of these cells in the intact brain. In order to provide more direct evidence for such **neuronal** influence, we attempted to induce, in the rat brain, a reversal of the post-lesional activation of astrocytes, microglia and endothelial cells by **transplantation** of fetal **neurons** purified by immunopanning. Host microglial cells which have been activated by the lesion process, penetrated the **neuronal** graft during the few days after the **transplantation** . Reactive astrocytes began to appear in the lesioned parenchyma and gathered around the **transplant** . Thereafter they first sent their processes in the direction of the **neuronal** graft, before they migrated into the graft a few days later. At this time, which was at the end of the first week post- **transplantation** , the host endothelial cells sprouted ''streamers'' of basal lamina within the graft forming small capillaries. During the second week post- **transplantation** , numerous astrocytes and microglial cells, both displaying a reactive hypertrophied morphology, were observed throughout the grafts. Finally, by the end of the first month, the activated cells differentiated towards a quiescent, resting morphology. At this time the grafts contained a vascular network with morphological characteristics comparable to those observed in the intact brain parenchyma.

The results indicate that the interaction of activated astroglia and microglia and endothelial cells with **neurons** causes the cells to re-differentiate and regain phenotypic features characteristic of intact brain parenchyma, strongly suggesting that **neurons** play an essential role in the phenotypic restriction of glial and endothelial cells in the adult central nervous system. (C) 1997 IBRO. Published by Elsevier Science Ltd.

17/3,AB/5 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05446196 Genuine Article#: VZ433 Number of References: 54
Title: LONG-TERM INTEGRATION AND NEURONAL **DIFFERENTIATION OF HUMAN**

EMBRYONAL CARCINOMA-CELLS (NTERA-2) TRANSPLANTED **INTO THE CAUDOPUTAMEN OF NUDE-MICE** (Abstract Available)
Author(s): MIYAZONO M; NOWELL PC; FINAN JL; LEE VMY; TROJANOWSKI JQ
Corporate Source: UNIV PENN,SCH MED,DEPT PATHOL & LAB MED,HUP MALONEY BASEMENT,ROOM A009,3600 SPRUCE ST/PHILADELPHIA//PA/19104; UNIV PENN,SCH MED,DEPT PATHOL & LAB MED/PHILADELPHIA//PA/19104
Journal: JOURNAL OF COMPARATIVE NEUROLOGY, 1996, V376, N4 (DEC 23), P 603-613
ISSN: 0021-9967
Language: ENGLISH Document Type: ARTICLE
Abstract: NTera-2 (NT2) cells are a human embryonal carcinoma (EC) cell line derived from a teratocarcinoma that differentiate exclusively into postmitotic **neurons** in vitro following retinoic acid (RA) treatment. Like other EC cell lines, NT2 cells rapidly form lethal tumors following **transplantation** into peripheral sites or many regions of the brain. However, when grafts are confined to the caudoputamen (CP), the NT2 cells differentiate into postmitotic **neuronlike** cells and do not form lethal tumors. To examine the long-term fate of such grafts, we studied NT2 cell **transplants** in the CP of nude mice that survived for > 1 year. NT2 cells in these grafts acquired molecular markers of fully mature **neurons** including the low, middle, and high molecular weight neurofilament proteins, microtubule-associated protein 2, tau, and synaptophysin. Furthermore, **neuronlike** cells in long-term CP grafts formed synaptic structures, and their processes became myelinated, whereas tyrosine hydroxylase (TH)-positive **neuronlike** cells in the grafts increased with progressively longer postimplantation survival times. Soluble extracts of the adult mouse CP augmented TH expression in RA-treated NT2 cells in vitro. These data suggest that the adult mouse CP is a source of factor(s) that inhibits tumor formation and induce a catecholaminergic **neuronal** phenotype in these human NT2 cells in vivo and in vitro. Identification of these factors could accelerate efforts to elucidate mechanisms that regulate progenitor cell fate and the commitment of **neurons** to specific neurotransmitter phenotypes. (C) 1996 Wiley-Liss, Inc.

17/3,AB/6 (Item 1 from file: 144)
DIALOG(R)File 144:Pascal

09202369 PASCAL No.: 90-0371551
Somatic activation of thalamic neurons transplanted **into lesioned somatosensory thalamus**
JULIANO S L; DUSART I; PESCHANSKI M
USUHS, dep. anatomy, Bethesda MD 20814, USA
Journal: Brain research, 1989, 478 (2) 356-360
Language: English
Des **neurones** homotypiques foetaux sont **transplantes** dans le complexe ventrobasal du thalamus du rat, prealablement lise. Par ce procede, les auteurs explorent le potentiel des connexions hote-greffe a **transporter** les inputs evoques par stimulation somatique peripherique en utilisant la technique du 2-desoxyglucose. Ils mettent egalement en evidence que les **neurones** greffes sont anatomiquement et fonctionnellement integres au SNC du rat hote

17/3,AB/7 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

00290270
IDENTIFYING NO.: 3P50AG05144-18S1 0011 AGENCY CODE: CRISP
CHOLINERGIC NEURONS **FOR** TRANSPLANTATION
PRINCIPAL INVESTIGATOR: HERSH, LOUIS B
ADDRESS: UNIVERSITY OF KENTUCKY 800 S LIMESTONE STREET LEXINGTON, KY 40536-0230
PERFORMING ORG.: UNIVERSITY OF KENTUCKY, LEXINGTON, KENTUCKY

SPONSORING ORG.: NATIONAL INSTITUTE ON AGING
FY : 2001

SUMMARY: The overall objective of this project is to obtain cells which can be used for **transplantation** to replace lost **neurons** in Alzheimer's and other neurodegenerative diseases involving cholinergic **neurons** . Two general approaches will be investigated; one involves transfecting genes of the key cholinergic proteins into cells capable of acquiring a **neuronal** phenotype, while the other involves the use of promoter elements from the ChAT gene to select out cholinergic **neurons** from fetal **neuronal** cell cultures containing cholinergic precursor cells. The project involves 4 specific aims. The first involves the use of cell transfection techniques to introduce the three major components of the cholinergic cell, the choline **transporter** , choline acetyltransferase, and the acetylcholine **transporter** into cells capable of acquiring a **neuronal** phenotype. These include NT-2 cells, RN33B cells, and embryonic **neuronal** precursor cells. Constructs containing the cholinergic specific genomic sequences will be used to express drug resistant genes in embryonic cholinergic **neuronal** precursor cells. Growth in the presence of the drug will be used to select for the cholinergic cells from this population. The second specific aim involves characterization of the transfected or isolated cells in terms of cholinergic function. This will focus on the ability of these cells to synthesize and store acetylcholine in synaptic vesicles, and to release acetylcholine upon depolarization. The ability of these cells to establish functional interactions with host **neurons** will initially be examined in vitro by co-culturing these cells with fetal brain tissue from various brain regions and looking for synaptic contacts. Thirdly, the transfected or isolated cells will be tested for their ability to survive intracerebral implantation in rats and to continue to synthesize, store, and secrete acetylcholine. Lastly, we will determine the ability of the implanted cells to ameliorate specific behavioral deficits in animal models of basal forebrain degeneration. It is anticipated that these studies will provide the basis for the further development of **neuronal** cells which can be used in gene replacement therapy for Alzheimer's and related diseases.

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